

STEREOSPECIFICITY OF LIPASES. ENZYMIC HYDROLYSIS OF ENANTIOMERIC ALKYL DIACYLGLYCEROLS BY LIPOPROTEIN LIPASE, LINGUAL LIPASE AND PANCREATIC LIPASE

F. PALTAUF*, F. ESFANDI and A. HOLASEK

*Institut für Biochemie, Technische Hochschule Graz,
and Institut für Medizinische Biochemie,
Universität Graz, Graz, Austria*

Received 14 December 1973

1. Introduction

There is general agreement that pancreatic lipase attacks both primary ester groups of triacylglycerol at equal rates, but is less active against the secondary position [1]. Conflicting results have been reported on the positional specificity of lipoprotein lipase. Korn [2] and Nilsson-Ehle et al. [3] found that lipoprotein lipase specifically cleaves the two primary ester bounds; Greten et al. [4], using dialkyl acylglycerols as substrates, concluded that the secondary ester was preferentially hydrolysed. Recently, Morley and Kuksis [5] presented evidence that lipoprotein lipase cleaves the ester in position 1 of *sn*-glycerol before the 2 and 3 positions are attacked.

In the present study racemic mixtures of alkyl diacylglycerols, consisting of equimolar amounts of 1-O-octadecyl-2,3-di-octadecenoyl-*sn*-glycerol (labeled with either ^3H in the alkyl moiety or with ^{14}C in both acyl groups) and 3-O-octadecyl-1,2-di-octadecenoyl-*sn*-glycerol (labeled with either ^{14}C in the alkyl moiety or ^3H in the acyl groups) were incubated with various lipases. Analysis of the isotope ratio found in the hydrolysis products (alkyl acylglycerols and alkylglycerols) suggests that lipoprotein lipase from human and rat post-heparin plasma, rat

adipose tissue and cow's milk preferentially hydrolyses the ester bound in the *sn*-1 position. Pancreatic lipase cleaves the ester bond in the *sn*-1 and *sn*-3 positions at equal rates. Lingual lipase cleaves the ester in position 3 twice as fast than in position 1. By these findings, the original observations of Morley and Kuksis [5], suggesting a stereospecificity for lipoprotein lipase, have been confirmed and extended. In addition, the results indicate that the naturally occurring 1-O-alkyl-2,3-diacyl-*sn*-glycerols are poor substrates for lipoprotein lipase, but are readily hydrolysed by pancreatic and lingual lipases.

The use of specifically labeled alkyl diacylglycerols provides a simple method for the stereospecificity assay of lipases.

2. Materials and methods

Radioactively labeled fatty acids and alcohols were purchased from the Radiochemical Centre, Amersham, England. 1-O-(9,10- $^3\text{H}_2$) octadecyl-*sn*-glycerol and the *sn*-3 isomer were prepared by catalytic tritiation of the corresponding octadecenyl derivatives [6]. The 3-O-octadecyl-*sn*-glycerol was prepared as described [6]. Alkyl-diacylglycerols and alkyl acylglycerols were synthesized by acylation of the corresponding alkyl glycerols with fatty acyl chlorides according to conventional procedures and the products purified by preparative TLC. Lipolytic activity in rat post-heparin plasma [7] was determined as follows: 1.8 μmoles of

* Mailing address: F. Paltauf, Institut für Biochemie, Technische Hochschule, Schlögelgasse 9, A-8010 Graz, Austria.

doubly labeled racemic alkyl diacylglycerol (see table 2), 0.1 ml 1% Triton X-100, 1 ml human serum (obtained after overnight fasting), 0.2 ml 1% bovine serum albumin (fatty acid-free, from Fluka, A.G., Buchs, Switzerland), 0.15 M Tris-HCl (pH 8.6), and 0.5 ml post-heparin plasma, in a total volume of 8 ml, were incubated with shaking at 37°C. Samples were withdrawn at times indicated and extracted with chloroform-methanol (2:1, v/v) (cf. ref. [6]). Respective lipid carriers were added and total lipids were fractionated by TLC employing light petroleum-diethyl ether-acetic acid (80:20:2, v/v/v) as the solvent system when substrates labeled in the acyl groups were used and chloroform-methanol-acetic acid (98:2:1, v/v/v) when substrates labeled in the alkyl moiety were employed. The radioactivity was determined by the use of a Packard Liquid Scintillation Spectrometer, Model 3315, set for double isotope counting. The enzyme specificity was determined from the ^3H or ^{14}C radioactivity present in the reaction products.

Lipolytic activities of lyophilized skim milk [8] and an acetone powder from rat epididymal adipose tissue [9] were assayed under similar conditions. Heparin (0.2 I.U.) was added to incubations when adipose tissue was employed. Lingual and pancreatic lipase were assayed according to Hamosh and Scow [9].

3. Results and discussion

According to Morley and Kuksis [5] the small amount of asymmetric (*sn*-1,2 plus *sn*-2,3) diglycerides isolated after incubation of triolein with lipoprotein lipase from post-heparin plasma or milk consists predominantly of the *sn*-2,3 species. This finding led to the conclusion that the hydrolytic process is initiated by cleavage in position 1 of the triglyceride. However, alternative explanations for this observation might be suggested. For example, a triglyceride lipase specific for both primary ester groups produces rac. 1,2 [2,3] diacylglycerol and the asymmetric diglycerides are then hydrolysed at unequal rates.

In order to avoid errors due to isomerization of hydrolysis products (di- and monoglycerides) Greten et al. [4] used acyl dialkylglycerols as substrates in studies on the positional specificity of lipoprotein

lipase. These authors found that the ester in position 2 was preferentially cleaved. The discrepancy between these results and the findings of others [2,3], that lipoprotein lipase hydrolysed the primary ester bonds more readily, might be explained by different physicochemical [3] and chemical properties for dialkyl acylglycerols as compared to triacylglycerols.

In the present study the enantiomeric alkyl diacylglycerols were used as substrates, which have one non-hydrolysable alkylether group in position 1 or 3; due to the presence of the vicinal ester groups in positions 1,2 or 2,3 these substrates resemble triglycerides more closely than do dialkyl acylglycerols. The stereospecific hydrolysis of a doubly labeled (^3H in the 1-alkyl and ^{14}C in the 3-alkyl moiety) racemic mixture of alkyl diacylglycerols by a lipase can easily be recognized by the enrichment of one of the isotopes in the reaction products. Incubation of both enantiomers in the same incubation vessel minimizes experimental errors due to differences in preparing substrate emulsions or incomplete extraction of products etc.

By this method it could be demonstrated that pancreatic lipase hydrolyses the ester bonds in position 3 of 1-alkyl-2,3 diacyl-*sn*-glycerol and in position 1 of 1,2-diacyl-3-alkyl-*sn*-glycerol at equal rates (table 1). These results are in agreement with results obtained with triacylglycerols [1,11]. Quite unexpectedly, only the *sn*-3 alkyl isomer was completely hydrolysed to alkylglycerol. Whether a similar stereospecific hydrolysis of 2-acyl-3-alkyl-*sn*-glycerol can also be observed when a purified preparation of pancreatic lipase is employed has not been established. An alternative explanation is the presence of another esterase in the crude pancreatin preparation.

The positional specificity of lingual lipase had not been established previously. As is shown in table 1, this enzyme cleaves both primary ester bonds. However, the ester bond in position 3 is cleaved at almost twice the rate as that in position 1. Lipoprotein lipase preferentially utilizes the enantiomer having an ester group in the 1 position (table 2). Results similar to those given in table 2 were obtained when 1-O-octadecyl-2,3-[1'- ^{14}C] octadecenoyl-*sn*-glycerol and 3-O-octadecyl-1,2-(9,10- $^3\text{H}_2$) dioctadecenoyl-*sn*-glycerol were used as substrates. In this case, [^3H] oleic acid was released and the ^{14}C -labeled substrate accumulated. These data clearly establish that hydrolysis of

Table 1

Enzymic hydrolysis of the enantiomeric 1(3)-O-alkyl-2,3 (1,2)-diacyl-*sn*-glycerol by pancreatic and lingual lipase. For incubation conditions see text.

	Source of enzyme						
	Pancreas*			Tongue**			
	0	5	10	0	5	10	20 minutes
Substrates	nanomoles						
1-O-Alkyl-2,3-diacyl- <i>sn</i> -glycerol***	1600	980	838	2000	1720	1558	1468
3-O-Alkyl-1,2-diacyl- <i>sn</i> -glycerol†	1600	997	880	2000	1834	1690	1638
Products							
1-O-Alkyl-2-acyl- <i>sn</i> -glycerol		580	710		240	358	436
3-O-Alkyl-2-acyl- <i>sn</i> -glycerol		480	580		128	234	254
1-O-Alkyl-3-acyl- <i>sn</i> -glycerol		32	38		20	50	64
3-O-Alkyl-1-acyl- <i>sn</i> -glycerol		38	30		12	46	60
1-O-Alkyl- <i>sn</i> -glycerol		8	14		18	36	36
3-O-Alkyl- <i>sn</i> -glycerol		85	110		22	28	46

* Pancreatin Merck, 3 mg per incubation.

** Homogenate prepared from the posterior part of the rat tongue, equivalent to 100 mg of wet tissue.

*** see footnote *, table 2.

† see footnote **, table 2.

trisubstituted glyceride by lipoprotein lipase proceeds via a stereospecific mechanism as originally suggested by Morley and Kuksis [5]. 1-Acyl-3-alkyl-*sn*-glycerol which would be formed by hydrolysis in position 2, does not accumulate to any appreciable degree (see table 2). This finding in combination with observations of others [2,3], that lipoprotein lipase attacks specifically the primary ester groups of triglycerides suggests that cleavage of the ester in position 1 is indeed the initial step during triglyceride hydrolysis by lipoprotein lipase. This assumption is corroborated by the fact that 2-acyl-3-alkyl-*sn*-glycerol, but no other alkyl acylglycerol is incorporated into alkyl diacylglycerol by the reverse action of lipoprotein lipase (table 3).

Hydrolysis of monoacyl alkylglycerol also appears

to be stereospecific, since the 1-alkyl-2-acyl isomer is almost completely resistant to hydrolysis whereas the 2-acyl-3-alkyl isomer is hydrolysed readily. With the 1(3)alkyl-3(1)acyl enantiomers differences in the reaction rates were less pronounced (table 3).

In summary it has been demonstrated that the stereospecificity of lipoprotein lipase is different from that of pancreatic and lingual lipases. The biological implications of the stereospecificity of lipoprotein lipase have been discussed [5] and it was suggested that the specificity of lipoprotein lipase results in the formation of 2,3-diacyl-*sn*-glycerol which cannot be reutilized for triglyceride or phosphoglyceride synthesis. In addition, the accumulating 2-monoglyceride may be reutilized in the adipose cell for triglyceride biosynthesis [12] or possibly play a

Table 2
Enzymic hydrolysis of the enantiomeric 1(3) alkyl-2,3(1,2) diacyl glycerols by lipoprotein lipase.

Substrates	Source of Enzyme								Adipose tissue
	Post-heparin serum				Milk				
	0	10	20	40	0	10	20	40	
	(minutes)				(minutes)				
nanomoles									
1-O-(³ H)-Alkyl-2,3-diacyl- <i>sn</i> -glycerol*	900	897	883	873	900	893	888	869	
3-O-(¹⁴ C)-Alkyl-1,2-diacyl- <i>sn</i> -glycerol**	900	873	827	798	900	834	801	764	
Products									
1-O-Alkyl-2-acyl- <i>sn</i> -glycerol		1.1	11.7	17.1		2.7	5.7	17.1	10.8
3-O-Alkyl-2-acyl- <i>sn</i> -glycerol		17.1	40.5	45.8		44.1	60.0	72.5	40.0
1-O-Alkyl-3-acyl- <i>sn</i> -glycerol		0	2.0	4.8		1.8	2.7	6.0	1.8
3-O-Alkyl-a-acyl- <i>sn</i> -glycerol		3.6	9.9	14.4		9.9	18.0	26.6	4.5
1-O-Alkyl- <i>sn</i> -glycerol		1.8	3.6	4.2		1.8	3.6	7.2	5.0
3-O-Alkyl- <i>sn</i> -glycerol		6.3	22.5	41.4		11.7	20.7	36.6	20.0

* 1-O-(9', 10'-³H₂)Octadecyl-2,3-dioctadecenoyl-*sn*-glycerol (specific radioactivity 10⁸ counts/min/mole).

** 3-O-(1'-¹⁴C)dioctadecyl-1,2-dioctadecenoyl-*sn*-glycerol (specific radioactivity 10⁸ counts/min/mole).

A mixture of 900 nmoles of each of both enantiomeric alkyl diacylglycerols*** was incubated as described under Materials and methods. The following amounts of enzyme preparations were used: 0.5 ml of rat post heparin serum; 40 mg of lyophilized skim milk [8], 4 mg of dry acetone powder from rat epididymal adipose tissue [9]. Each experiment was carried out at least three times with essentially results (deviation was less than 5%).

Table 3
Enzymic hydrolysis of the enantiomers, 1(3)-O-alkyl-2-acyl *sn*-glycerol*, and 1(3)-O-alkyl-3(1)-acyl-*sn*-glycerol** by lipoprotein lipase from milk.

Substrates	Products (nmoles)					
	Fatty acid			Alkyl diacylglycerol		
	10	20	40 min.	10	20	40 min.
1-O-Alkyl-2-acyl- <i>sn</i> -glycerol*			3.0	0.0	0.0	0.0
3-O-Alkyl-2-acyl- <i>sn</i> -glycerol*	10.4	16.2	30.0***	5.8	8.6	14.4***
1-O-Alkyl-3-acyl- <i>sn</i> -glycerol**		25.0	50.0			0.0
3-O-Alkyl-1-acyl- <i>sn</i> -glycerol**	48.9	73.4	96.4			0.0

200 nmoles of the respective substrate were incubated in the presence of a suspension of 50 mg of lyophilized skim milk as described under Materials and methods.

* 1(3)-O-octadecyl-2-(9', 10'-³H₂) octadecenoyl-*sn*-glycerol (spec. radioactivity 2.10⁶ counts/min/μmole).

** 1(3)-O-octadecyl-3(1)-(9', 10'-³H₂) octadecenoyl-*sn*-glycerol (spec. radioactivity 2.10⁶ counts/min/μmole).

*** In a separate experiment, employing 2-octadecenoyl-3-(1'-¹⁴C)-octadecyl-*sn*-glycerol as the substrate, 23 nmoles of 3-O-alkyl glycerol were released after 40 min; 7 nmoles were incorporated into alkyl-diacylglycerol.

role in regulating glyceride synthesis via the glycerol-3-phosphate pathway [13].

The 1-alkyl-2,3-diacyl-*sn*-glycerols are naturally occurring lipids [14] which, according to present results, are quite resistant to hydrolysis by lipoprotein lipase. The importance of these observations in biological systems is at present unknown.

References

- [1] Borgström, B. (1964) *J. Lipid Res.* 5, 522–531.
- [2] Korn, E.D. (1962) *J. Lipid Res.* 3, 246–250.
- [3] Nilsson-Ehle, P., Belfrage, P. and Borgstrom, B. (1971) *Biochim. Biophys. Acta* 248, 114–120.
- [4] Greten, H., Levy, R.I., Fales, H. and Fredrickson, D.S. (1970) *Biochim. Biophys. Acta* 210, 39–45.
- [5] Morley, N. and Kuksis, A. (1972) *J. Biol. Chem.* 247, 6389–6393.
- [6] Paltauf, F. (1971) *Biochim. Biophys. Acta* 239, 38–46.
- [7] Fielding, C.J. (1968) *Biochim. Biophys. Acta* 159, 94–102.
- [8] Bier, D.M. and Havel, R.J. (1970) *J. Lipid Res.* 11, 565–570.
- [9] Robinson, D.S. (1963) *J. Lipid Res.* 4, 21–23.
- [10] Hamosh, H. and Scow, R.O. (1973) *J. Clin. Invest.* 52, 88–95.
- [11] Brockerhoff, H. (1965) *J. Lipid Res.* 6, 10–15.
- [12] Schultz, F.M. and Johnston, J.M. (1971) *J. Lipid Res.* 12, 132–138.
- [13] Polheim, D., David, J.S.K., Schultz, F.M., Wylie, M.B. and Johnston, J.M. (1973) *J. Lipid Res.* 14, 415–421.
- [14] Horrocks, L.A. (1972) in: *Ether Lipids, Chemistry and Biology* (F. Snyder, ed.), pp. 180–183, Academic Press, New York and London.